

Claims

1. A method for analyzing a polymer comprising
contacting the polymer with a conjugate comprising a nucleic acid tag molecule and a
nucleic acid binding agent,
5 allowing the nucleic acid binding agent to bind to the polymer non-specifically, and
allowing the nucleic acid tag molecule to bind specifically to the polymer, and
determining a pattern of binding of the conjugate to the polymer.
2. The method of claim 1, further comprising allowing the nucleic acid binding
10 agent to translocate along the polymer.
3. The method of claim 1, wherein the nucleic acid binding agent binds to the
polymer non-specifically.
- 15 4. The method of claim 1, wherein the polymer is a nucleic acid molecule.
5. The method of claim 1, wherein the polymer is DNA or RNA.
6. The method of claim 1, wherein the nucleic acid tag molecule is selected from
20 the group consisting of a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a DNA, an
RNA, a bisPNA clamp, a pseudocomplementary PNA, and a LNA-DNA co-polymer.
7. The method of claim 1, wherein the nucleic acid tag molecule is 5-50 residues
in length.
25
8. The method of claim 1, wherein the nucleic acid tag molecule and the nucleic
acid binding agent are covalently linked to each other.
9. The method of claim 1, wherein the nucleic acid tag molecule and the nucleic
30 acid binding agent are conjugated using a linker molecule.
10. The method of claim 1, wherein the nucleic acid binding agent is an enzyme.

11. The method of claim 10, wherein the enzyme is selected from the group consisting of a DNA polymerase, an RNA polymerase, a DNA repair enzyme, a helicase, a nuclease, and a ligase.

5 12. The method of claim 10, wherein the enzyme lacks the ability to modify the nucleic acid tag molecule or the polymer.

13. The method of claim 1, wherein the nucleic acid tag molecule is labeled with a detectable moiety.

10

14. The method of claim 1, wherein the nucleic acid binding agent is labeled with a detectable moiety.

15 15. The method of claim 1, wherein the nucleic acid tag molecule is labeled with a first detectable moiety, and the nucleic acid binding agent is labeled with a second detectable moiety.

16. The method of claim 1, wherein the polymer is labeled with a detectable moiety.

20

17. The method of claim 16, wherein the detectable moiety is a backbone specific label.

18. The method of claim 1, wherein the nucleic acid binding agent is not itself a
25 detectable moiety.

19. The method of claim 1, wherein the pattern of binding of the conjugate to the polymer is determined using a linear polymer analysis system.

30 20. The method of claim 19, wherein the linear polymer analysis system comprises exposing the polymer to a station to produce a signal arising from the binding of the conjugate to the polymer, and detecting the signal using a detection system.

21. The method of claim 1, wherein the pattern of binding of the conjugate to the polymer is determined using fluorescence in situ hybridization (FISH).

22. The method of claim 13, 14, or 15, wherein the detectable moiety is selected
5 from the group consisting of an electron spin resonance molecule, a fluorescent molecule, a chemiluminescent molecule, a radioisotope, an enzyme substrate, a biotin molecule, an avidin molecule, an electrical charged transferring molecule, a semiconductor nanocrystal, a semiconductor nanoparticle, a colloid gold nanocrystal, a ligand, a microbead, a magnetic bead, a paramagnetic particle, a quantum dot, a chromogenic substrate, an affinity molecule, a
10 protein, a peptide, a nucleic acid, a carbohydrate, an antigen, a hapten, an antibody, an antibody fragment, and a lipid.

23. The method of claim 22, wherein the detectable moiety is detected using a detection system selected from the group consisting of an electron spin resonance detection
15 system, a charge coupled device (CCD) detection system, a fluorescent detection system, an electrical detection system, a photographic film detection system, a chemiluminescent detection system, an enzyme detection system, an atomic force microscopy (AFM) detection system, a scanning tunneling microscopy (STM) detection system, an optical detection system, a nuclear magnetic resonance (NMR) detection system, a near field detection system,
20 and a total internal reflection (TIR) detection system.

24. The method of claim 1, wherein the polymer is a non in vitro amplified nucleic acid molecule.

25. The method of claim 1, wherein the nucleic acid tag molecule is not an
25 antisense molecule.

26. The method of claim 1, wherein the nucleic acid tag molecule does not hybridize to bacterial or viral specific sequences.
30

27. The method of claim 1, wherein the nucleic acid tag molecule is labeled with an agent.

28. The method of claim 27, wherein the agent is capable of cleaving a nucleic acid molecule.

29. The method of claim 28, wherein the agent is a photocleaving agent.

5

30. The method of claim 27, wherein the agent is able to modify a nucleic acid molecule.

31. The method of claim 1, wherein the nucleic acid binding agent is detected indirectly.

10

32. The method of claim 31, wherein the nucleic acid binding agent is detected indirectly using an antibody or an antibody fragment specific for the nucleic acid binding agent.

15

33. The system of claim 19, wherein the linear polymer analysis system is a single polymer analysis system.

34. The system of claim 1, wherein the pattern of binding of the conjugate to the polymer is determined using a method selected from the group consisting of Gene Engine™, optical mapping, and DNA combing.

20

35. A system for optically analyzing a polymer comprising:
an optical source for emitting optical radiation;
an interaction station for receiving the optical radiation and for receiving a polymer that is exposed to the optical radiation to produce detectable signals; and
a processor constructed and arranged to analyze the polymer based on the detected radiation including the signals,
wherein the polymer is bound to a conjugate comprising a nucleic acid tag molecule and a nucleic acid binding agent.

25

30

36. The system of claim 35, wherein the polymer is a nucleic acid molecule.

37. The system of claim 35, wherein the polymer is DNA or RNA.

38. The system of claim 35, wherein the nucleic acid tag molecule of the conjugate is selected from the group consisting of a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a DNA, an RNA, a bisPNA clamp, a pseudocomplementary PNA, and a LNA-DNA co-polymer.

39. The system of claim 35, wherein the nucleic acid tag molecule is 5-50 residues in length.

10

40. The system of claim 35, wherein the nucleic acid tag molecule and the nucleic acid binding agent are covalently conjugated to each other.

41. The system of claim 35, wherein the nucleic acid tag molecule and the nucleic acid binding agent are conjugated to each other using a linker molecule.

15

42. The system of claim 35, wherein the nucleic acid binding agent is an enzyme.

43. The system of claim 42, wherein the enzyme is selected from the group consisting of a DNA polymerase, an RNA polymerase, a DNA repair enzyme, a helicase, a nuclease, and a ligase.

20

44. The system of claim 42, wherein the enzyme lacks the ability to modify the nucleic acid tag molecule or the polymer.

25

45. The system of claim 35, wherein the nucleic acid tag molecule is labeled with a detectable moiety.

46. The system of claim 35, wherein the nucleic acid binding agent is labeled with a detectable moiety.

30

47. The system of claim 35, wherein the nucleic acid tag molecule is labeled with a first detectable moiety, and the nucleic acid binding agent is labeled with a second detectable moiety.

5 48. The system of claim 35, wherein the polymer is labeled with a detectable moiety.

49. The system of claim 48, wherein the detectable label is a backbone specific label.

10

50. The system of claim 45, 46, or 47, wherein the detectable moiety is selected from the group consisting of an electron spin resonance molecule, a fluorescent molecule, a chemiluminescent molecule, a radioisotope, an enzyme substrate, a biotin molecule, an avidin molecule, an electrical charged transferring molecule, a semiconductor nanocrystal, a
15 semiconductor nanoparticle, a colloid gold nanocrystal, a ligand, a microbead, a magnetic bead, a paramagnetic particle, a quantum dot, a chromogenic substrate, an affinity molecule, a protein, a peptide, a carbohydrate, an antibody, an antibody fragment, an antigen, a hapten, and a lipid.

20 51. The system of claim 50, wherein the detectable moiety is detected using a detection system selected from the group consisting of a charge coupled device (CCD) detection system, an electron spin resonance detection system, a fluorescent detection system, an electrical detection system, a photographic film detection system, a chemiluminescent detection system, an enzyme detection system, an atomic force microscopy (AFM) detection
25 system, a scanning tunneling microscopy (STM) detection system, an optical detection system, a nuclear magnetic resonance (NMR) detection system, a near field detection system, and a total internal reflection (TIR) detection system.

52. The system of claim 35, wherein the polymer is a non in vitro amplified
30 nucleic acid molecule.

53. The system of claim 35, wherein the interaction station includes a slit having a slit width in a range of 1 nm to 500 nm and producing a localized radiation spot.

54. The system of claim 53, wherein the slit width is in a range of 10 nm to 100 nm.

5 55. The system of claim 53, further comprising a microchannel arranged with the slit to produce the localized radiation spot, the microchannel being constructed to receive and advance the polymer through the localized radiation spot.

10 56. The system of claim 53, further comprising a polarizer, wherein the optical source includes a laser constructed to emit a beam of radiation and the polarizer is arranged to polarize the beam prior to reaching the slit.

15 57. The system of claim 56, wherein the polarizer is arranged to polarize the beam parallel to the width of the slit.

58. The system of claim 35, further comprising a microchannel arranged to produce a localized radiation spot, the microchannel being constructed to receive and advance the polymer through the localized radiation spot.

20 59. The system of claim 35, further comprising a polarizer, wherein the optical source includes a laser constructed to emit a beam of radiation and the polarizer is arranged to polarize the beam.

25 60. The system of claim 35, wherein the optical source is a light source integrated on a chip.

61. The system of claim 35, wherein the conjugate of the nucleic acid tag molecule and the nucleic acid binding agent is specifically bound to the polymer.

30 62. The system of claim 35, wherein the nucleic acid binding agent is bound non-specifically to the polymer.

63. The system of claim 35, wherein the nucleic acid binding agent is detected indirectly.

5 64. The system of claim 63, wherein the nucleic acid binding agent is detected indirectly using an antibody or an antibody fragment specific for the nucleic acid binding agent.

65. The system of claim 51 wherein the detection system is incorporated into a linear polymer analysis system.
10

66. The system of claim 65, wherein the linear polymer analysis system is a single polymer analysis system.

67. The system of claim 35, wherein the polymer is analyzed using a method
15 selected from the group consisting of Gene Engine™, optical mapping, and DNA combing.

68. A method for analyzing a polymer comprising:
generating optical radiation of a known wavelength to produce a localized radiation spot;
20 passing a polymer through a microchannel;
irradiating the polymer at the localized radiation spot;
sequentially detecting radiation resulting from interaction of the polymer with the optical radiation at the localized radiation spot; and
analyzing the polymer based on the detected radiation,
25 wherein the polymer is bound to a conjugate of a nucleic acid tag molecule and a nucleic acid binding agent.

69. The method of claim 68, wherein the polymer is a nucleic acid molecule.

30 70. The method of claim 69, further comprising employing an electric field to pass the nucleic acid molecule through the microchannel.

71. The method of claim 69, wherein the detecting includes collecting the signals over time while the nucleic acid molecule is passing through the microchannel.

5 72. The method of claim 68, wherein the nucleic acid tag molecule of the conjugate binds specifically to the polymer and the nucleic acid binding agent binds non-specifically to the polymer.

73. The method of claim 69, wherein the nucleic acid molecule is DNA or RNA.

10 74. The method of claim 68, wherein the nucleic acid molecule of the conjugate is selected from the group consisting of a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a DNA, an RNA, a bisPNA clamp, a pseudocomplementary PNA, and a LNA-DNA co-polymer.

15 75. The method of claim 69, wherein the nucleic acid molecule is 5-50 residues in length.

76. The method of claim 68, wherein the nucleic acid tag molecule and the nucleic acid binding agent are covalently conjugated to each other.

20

77. The method of claim 68, wherein the nucleic acid molecule and the nucleic acid binding agent are conjugated to each other using a linker molecule.

78. The method of claim 68, wherein the nucleic acid binding agent is an enzyme.

25

79. The method of claim 78, wherein the enzyme is selected from the group consisting of a DNA polymerase, an RNA polymerase, a DNA repair enzyme, a helicase, a nuclease, and a ligase.

30 80. The method of claim 78, wherein the enzyme lacks the ability to modify a nucleic acid molecule.

81. The method of claim 68, wherein the nucleic acid tag molecule is labeled with a detectable moiety.

82. The method of claim 68, wherein the nucleic acid binding agent is labeled with
5 a detectable moiety.

83. The method of claim 68, wherein the nucleic acid molecule is labeled with a first detectable moiety, and the nucleic acid binding agent is labeled with a second detectable moiety.

10

84. The method of claim 68, wherein the polymer is labeled with a detectable moiety.

85. The method of claim 84, wherein the detectable moiety is a backbone specific
15 label.

86 The method of claim 81, 82, or 83, wherein the detectable moiety is selected from the group consisting of an electron spin resonance molecule, a fluorescent molecule, a chemiluminescent molecule, a radioisotope, an enzyme substrate, a biotin molecule, an avidin
20 molecule, an electrical charged transferring molecule, a semiconductor nanocrystal, a semiconductor nanoparticle, a colloid gold nanocrystal, a ligand, a microbead, a magnetic bead, a paramagnetic particle, a quantum dot, a chromogenic substrate, an affinity molecule, a protein, a peptide, nucleic acid, a carbohydrate, an antigen, a hapten, an antibody, an antibody fragment, and a lipid.

25

87. The method of claim 86, wherein the detectable moiety is detected using a detection system selected from the group consisting of an electron spin resonance detection system, a charge coupled device detection system, a fluorescent detection system, an electrical detection system, a photographic film detection system, a chemiluminescent detection system,
30 an enzyme detection system, an atomic force microscopy (AFM) detection system, a scanning tunneling microscopy (STM) detection system, an optical detection system, a nuclear magnetic resonance (NMR) detection system, a near field detection system, and a total internal reflection (TIR) detection system.

88. The method of claim 69, wherein the nucleic acid is a non in vitro amplified nucleic acid molecule.

5 89. The method of claim 68, wherein the nucleic acid binding agent is detected indirectly.

90. The method of claim 89, wherein the nucleic acid binding agent is detected indirectly using an antibody or an antibody fragment specific for the nucleic acid binding
10 agent.

91. A method for analyzing a nucleic acid molecule, comprising:
exposing a nucleic acid molecule to a conjugate of a nucleic acid tag molecule and a
nucleic acid binding enzyme,
15 allowing the nucleic acid binding enzyme to bind to the nucleic acid molecule,
allowing the nucleic acid tag molecule to bind to the nucleic acid molecule in a
sequence-specific manner, and
determining a pattern of binding of the conjugate to the nucleic acid molecule.

20 92. The method of claim 91, wherein the nucleic acid binding enzyme binds to the nucleic acid molecule non-specifically.

93. The method of claim 91, wherein the nucleic acid molecule is DNA or RNA.

25 94. The method of claim 91, wherein the nucleic acid tag molecule is selected from the group consisting of a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a DNA, an RNA, a bisPNA, a pseudocomplementary PNA, and a LNA-DNA co-polymer.

95. The method of claim 91, wherein the nucleic acid tag molecule is 5-50 residues
30 in length.

96. The method of claim 91, wherein the nucleic acid tag molecule and the nucleic acid binding enzyme are covalently linked to each other.

97. The method of claim 91, wherein the nucleic acid tag molecule and the nucleic acid binding enzyme are conjugated to each other using a linker.

5 98. The method of claim 91, wherein the nucleic acid binding enzyme is selected from the group consisting of a DNA polymerase, an RNA polymerase, a DNA repair enzyme, a helicase, a nuclease, and a ligase.

99. The method of claim 98, wherein the enzyme nucleic acid binding lacks the
10 ability to modify the nucleic acid molecule or the tag molecule.

100. The method of claim 91, wherein the nucleic acid tag molecule is labeled with a detectable moiety.

15 101. The method of claim 91, wherein the nucleic acid binding enzyme is labeled with a detectable moiety.

102. The method of claim 91, wherein the nucleic acid tag molecule is labeled with a first detectable moiety, and the nucleic acid binding enzyme is labeled with a second
20 detectable moiety.

103. The method of claim 91, wherein the nucleic acid molecule is labeled with a detectable moiety.

25 104. The method of claim 91, wherein the nucleic acid molecule is labeled with a backbone specific label.

105. The method of claim 91, wherein the pattern of binding of the conjugate to the nucleic acid molecule is determined using a linear nucleic acid analysis system.

30

106. The method of claim 105, wherein the linear nucleic acid analysis system comprises exposing the polymer to a station to produce a signal arising from the binding of the conjugate to the polymer, and detecting the signal using a detection system.

107. The method of claim 100, 101, or 102, wherein the detectable moiety is selected from the group consisting of an electron spin resonance molecule, a fluorescent molecule, a chemiluminescent molecule, a radioisotope, an enzyme substrate, a biotin molecule, an avidin molecule, an electrical charged transferring molecule, a semiconductor nanocrystal, a semiconductor nanoparticle, a colloid gold nanocrystal, a ligand, a microbead, a magnetic bead, a paramagnetic bead, a quantum dot, a chromogenic substrate, an affinity molecule, a protein, a peptide, a nucleic acid, a hapten, an antigen, an antibody, an antibody fragment, a carbohydrate, and a lipid.

10

108. The method of claim 107, wherein the detectable moiety is detected using a detection system selected from the group consisting of an electron spin resonance detection system, a charge coupled device detection system, a fluorescent detection system, an electrical detection system, a photographic film detection system, a chemiluminescent detection system, an enzyme detection system, an atomic force microscopy (AFM) detection system, a scanning tunneling microscopy (STM) detection system, an optical detection system, a nuclear magnetic resonance (NMR) detection system, a near field detection system, and a total internal reflection (TIR) system.

20

109. The method of claim 91, wherein the nucleic acid molecule is a non in vitro amplified nucleic acid molecule.

110. The method of claim 91, wherein the nucleic acid binding agent is detected indirectly.

25

111. The method of claim 110, wherein the nucleic acid binding agent is detected indirectly using an antibody or an antibody fragment specific for the nucleic acid binding agent.

30

112. A composition comprising a conjugate of a nucleic acid tag molecule and a nucleic acid binding enzyme, wherein a detectable moiety is present on the nucleic acid binding enzyme.

113. A composition comprising
a conjugate of a nucleic acid tag molecule and a nucleic acid binding enzyme,
wherein a detectable moiety is present on the nucleic acid tag molecule and wherein
the nucleic acid binding enzyme is not the detectable moiety.

5

114. The composition of claim 112 or 113, wherein the nucleic acid tag molecule
and the nucleic acid binding agent are covalently linked to each other.

115. The composition of claim 112 or 113, wherein the nucleic acid tag molecule
10 and the nucleic acid binding agent are linked to each other using a linker molecule.

116. The composition of claim 112 or 113, wherein the nucleic acid tag molecule is
selected from the group consisting of a peptide nucleic acid (PNA), a locked nucleic acid
(LNA), a DNA, an RNA, a bisPNA clamp, a pseudocomplementary PNA, and a LNA-DNA
15 co-polymer.

117. The composition of claim 112 or 113, wherein the nucleic acid binding enzyme
is selected from the group consisting of a DNA polymerase, an RNA polymerase, a DNA
repair enzyme, a helicase, a nuclease, and a ligase.

20

118. The composition of claim 112 or 113, wherein the nucleic acid binding enzyme
lacks the ability to modify a nucleic acid molecule.

119. The composition of claim 112, wherein the nucleic acid tag molecule is labeled
25 with a second detectable moiety.

120. The composition of claim 113, wherein the nucleic acid binding enzyme is
labeled with a second detectable moiety.

121. The composition of claim 112, 113, 119 or 120, wherein the detectable moiety
30 is selected from the group consisting of an electron spin resonance molecule, a fluorescent
molecule, a chemiluminescent molecule, a radioisotope, an enzyme substrate, a biotin
molecule, an avidin molecule, an electrical charged transferring molecule, a semiconductor

nanocrystal, a semiconductor nanoparticle, a ligand, a microbead, a magnetic bead, a paramagnetic molecule, a quantum dot, a chromogenic substrate, an affinity molecule, a protein, a peptide, nucleic acid, a carbohydrate, a hapten, an antigen, an antibody, an antibody fragment, and a lipid.

5

122. The composition of claim 121, wherein the detectable moiety is detected using a detection system selected from the group consisting of an electric spin resonance detection system, a charge coupled device detection system, a fluorescent detection system, an electrical detection system, a photographic film detection system, a chemiluminescent detection system,
10 an enzyme detection system, an atomic force microscopy (AFM) detection system, a scanning tunneling microscopy (STM) detection system, an optical detection system, a nuclear magnetic resonance (NMR) detection system, a near field detection system, and a total internal reflection (TIR) system.

15

123. The method of claim 112, wherein the nucleic acid binding agent is detected indirectly.

20

124. The method of claim 123, wherein the nucleic acid binding agent is detected indirectly using an antibody or an antibody fragment specific for the nucleic acid binding agent.

25

125. A method for analyzing a polymer comprising
contacting the polymer with a conjugate comprising a nucleic acid tag molecule and a nucleic acid binding agent,
allowing the nucleic acid binding agent to bind to the polymer, and
allowing the nucleic acid tag molecule to bind specifically to the polymer,
wherein the nucleic acid binding agent is selected from the group consisting of a DNA repair enzyme, a helicase, a nuclease, and a ligase.

30

126. A method for labeling a polymer comprising
contacting the polymer with a conjugate comprising a nucleic acid tag molecule and a nucleic acid binding agent,

allowing the nucleic acid binding agent to bind to and translocate along the polymer, and

allowing the nucleic acid tag molecule to bind specifically to the polymer.

5 127. The method of claim 126, wherein the nucleic acid binding agent binds to the polymer non-specifically.

 128. The method of claim 126, further comprising determining a pattern of binding of the conjugate to the polymer.